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Anti-N SARS-CoV-2 assays for evaluation of natural viral infection

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ABSTRACT

Background: The 2019 coronavirus (COVID-19) epidemic, required the development of different diagnostic tests. While reverse transcriptase real-time PCR (RT-PCR) remains the first-line test of choice in acute infection diagnosis, anti-N antibodies serological assays provide a valuable tool to differentiate natural SARS-CoV-2 immunological response from that induced by vaccination, thus the goal of our study was to evaluate three serological tests agreement for these antibodies detection.
Methods: Three anti-N different tests were examined in 74 sera from patients referred or not COVID infection: immunochromatographic rapid test (Panbio™ COVID-19 IgG/IgM Rapid Test Device Abbott, Germany), ELISA kit (NovaLisa® SARS-CoV-2 IgG and IgM NovaTech Immunodiagnostic GmbH, Germany) and ECLIA immunoassay (Elecsys® Anti-SARS-CoV-2 Roche Diagnostics, Manheim, Germany).
Results: Qualitative comparison of the three analytical methods revealed a moderate agreement between ECLIA immunoassay and immunochromatographic rapid test (Cohen kappa coefficient $\kappa = 0.564$). Correlation analysis indicated weak positive correlation between total Ig (IgT) detected by ECLIA immunoassay and IgG by ELISA test ($p < 0.0001$), the analysis of ECLIA IgT and IgM ELISA detected, showed no statistical correlation.
Conclusion: Comparison between of three analytical systems available for anti-N SARS-CoV-2 IgG and IgM antibodies showed a general agreement when compared to detect total and G class immunoglobulins, while doubtful or discordant results have been highlighted for IgT and IgM class. Anyway, all the tests examined provide reliable results to assess the serological status of SARS-CoV-2 infected patients.

1. Introduction

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), the causative agent of COVID-19, is a highly transmissible enveloped virus with a positive single-stranded RNA genome (Hu et al., 2021). SARS-CoV-2 belong to the Coronaviridae family, sharing with them common structural and biological properties. Four structural viral proteins named spike (S), envelope (E), membrane (M), and nucleocapsid (N)) have been identified as necessary for virion assembly and suppression of the host immune response (Yang and Rao, 2021; Su et al., 2016; Zhu et al., 2020). COVID-19 is characterized by several symptoms ranging from a mild common cold, cough and fever, to severe respiratory distress and multi-organ failure that leads to death (Knoll et al., 2021). In these conditions, it is likely that the decision to refer a patient to hospital depended mainly on the severity of the clinical picture or on how suggestive it was of SARS-CoV-2. Emerging evidence suggests that severity and poor prognosis in COVID-19 patients could be related to an excessive response of the immune system, mainly characterized by the abnormal release of circulating cytokines and by unusual biochemical parameters observed in these patients (Anastasi et al., 2022; Anastasi et al., 2020; Gandini et al., 2020; Antonelli et al., 2021). The incubation time from exposure to symptom onset commonly is from 2 to 14 days (Chan et al., 2020; Lauer et al., 2020). The viral load of SARS-CoV-2 achieves the peak and it's usually detectable with the onset of clinical symptoms, although most patients remain asymptomatic (Walsh et al., 2020; Zhou et al., 2020; Mizumoto et al., 2020). To date, probe-based reverse transcriptase real-time PCR (RT-PCR) detecting viral RNA is the current reference standard diagnostic tool to detect ongoing SARS-

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CoV-2 infection (Rai et al., 2021). Reverse transcription-polymerase chain reaction (RT-PCR) remains the gold standard for diagnosing COVID-19, and genomic sequencing has become vital for tracking variants. However, lateral flow tests (LFTs), albeit less sensitive than PCR, have enabled an unprecedented scale of global testing in clinical and public health, owing to their simplicity, low cost, accessibility, rapid results and ability to detect infectiousness (Budd et al., 2023). Serological tests are elective methods to assay seroconversion and epidemiology of SARS-CoV-2 and will represent an important instrument to track immune response promoted by future vaccine against this virus. Tools for the diagnosis of SARS-CoV-2 infection have become pivotal in the efforts to control the infection and monitor infected subjects (Van Caeseele et al., 2020). Three classes of immunoglobulins (IgM, IgG and IgA) against S and N antigens are usually detectable in immune response to the infection while the response to mRNA vaccines is demonstrated by the presence of anti-S antibodies alone (Okba et al., 2020; Zhao et al., 2020; Deeks et al., 2020; Weinstein et al., 2020). Even though the high sensitivity of serological tests (ranging from 96.0% to 97.8%), to date there is no evidence supporting the use of antibody tests in management of COVID-19 patients (Deeks et al., 2020; Chen et al., 2021). Currently, many efforts are employed to develop high specificity and sensitivity tests to identify seroconversion timing (Deeks et al., 2020). Infected patients show antibodies against a wide variety of viral proteins including the anti-N and anti-S antibodies (Mueller, 2021). The N protein is associated with the viral genome and produced in large quantities in early stages of the infection. Due to the high specificity of the N protein, no cross-reactivity with other related viruses is observed; for this reason, the antibody tests for the N protein are highly specific especially in the diagnosis of COVID-19 infection. The N protein is exclusively an index of natural infection, but the S protein can be an index not only of infection, but also of vaccination (Uysal et al., 2022). Due the high prevalence of COVID-19 as reported by several epidemiological studies, it's important to have an analytical method for the appropriate identification of symptomatic and or asyntomatic infected patients (Zhou et al., 2021; Azami et al., 2022).

Several tests are available for the detection of SARS-CoV-2 anti-N immunoglobulins (IgG, IgM and IgA) that reveal different sensitivities and specificities (Böger et al., 2021). In laboratory medicine, automation is considered one of the most important breakthroughs in diagnostics since it improves efficiency, accuracy, reproducibility, standardization, quality and safety. Moreover, human errors, analysis reporting time and costs are drastically reduced (Weinstein et al., 2020). Several anti-N SARS-CoV-2 kits based on different analytical approach (i.e immunochromatographic, ECLIA, ELISA) are available, but these systems report contrasting results highlighting the need to compare the different methods (Lippi and Da Rin, 2019; Ferraro et al., 2016). Thus, we aimed to compare three different analytical assays for the measurement of anti-N SARS-CoV-2 antibodies.

2. Materials and method

2.1. Sera

Seventy four sera from 70 adult patients (49 male and 21 female with the age ranging from 30 to 70 years) were enrolled in the study, for 3 patients sera samples were collected at different times. Sera were obtained from various outpatient services of the “Azienda Ospedaliera Policlinico Umberto I" Sapienza University of Rome from March 2021 to March 2022. Twenty-nine (41%) patients Sars-cov2 infected and documented by positivity of molecular swab between 1 month and 1 year before to sampling, 41 (59%) patients had no evidence of known SARS-CoV-2 infection and relative symptoms in the previous year.

All sera were acquired following a standard protocol. Briefly, samples were collected in serum separator tubes (Becton, Dickinson and Co., Plymouth UK) clotted 60–90 min and centrifuged for 10 min at 1300 $\times g$. The serum fractions obtained were then stored in 1.5 ml Eppendorf tubes (Eppendorf S.r.l., Milano Italy) and stored at −20 °C until analysis.

This research was reviewed and approved by the ethical committee of Azienda Ospedaliera Universitaria Policlinico Umberto I (ClinicalTrials.gov Identifier: NCT04844632).

2.2. Methods

Anti-N SARS-CoV-2 antibody testing was performed in two different laboratories: Virology Unit and Tumour Markers laboratory of the “Azienda Ospedaliera Policlinico Umberto I" La Sapienza University of Rome. Sera specimens were tested according to the manufacturers' instructions as follows.

Panbio™ COVID-19 IgG/IgM Rapid Test Device (Abbott, Germany) is an immunochromatographic rapid test for the qualitative detection of IgG and IgM antibodies to SARS-CoV-2 in human serum, plasma, venous and fingerstick whole blood. A valid result consists in the appearance of a red line in the C (Control) area of the reading window. A negative result consists in only the red line in the C area. The presence of a red line also in G (IgG) and/or M (IgM) areas of the reading window indicates the presence of IgG/IgM. As reported by the manufacturing company the assay shows a sensitivity of 97.8% with 95% confidence interval (92.1%–99.7%) and a specificity of 92.8% with 95% confidence interval of (88.9%–95.7%).

NovaLisa® SARS-CoV-2 IgG and IgM (Nova Tech Immunodiagnostic GmbH, Germany) is an Enzyme-Linked Immunosorbent Assays (ELISA) intended for the separate qualitative determination of IgG and IgM antibodies against SARS-CoV-2 Nucleocapsid (N) protein in human serum or plasma (citrate, heparin). This assay is based on the use of microtiter plates coated with specific antigens to bind corresponding antibodies of the sample. This method uses horseradish peroxidase (HRP)-labeled conjugate and tetramethylbenzidine (TMB) as detection agents. The final absorbance is performed at 450–620 nm using an ELISA microtiter plate reader. The results are expressed in NTU (NovaTec Units) which are obtained by multiplying the sample absorbance value by 10 and dividing the result by the cut-off. The cut-off is the mean absorbance value of the cut-off control determinations. Values >11 NTU are considered positive, values between 9 and 11 NTU are equivocal and < 9 NTU are negative. Manufacturer reports that NovaLisa® SARS-CoV-2 IgG and IgM provides positive results with a specificity of 98.76% (95%- confidence interval: 95.58% - 99.85%) and a 100% sensitivity in samples obtained >12 days post symptom onset.

Elecsys® Anti-SARS-CoV-2 (Roche Diagnostics, Manheim, Germany) is an established electrochemiluminescent immunoassay (ECLIA) targeting immunoglobulin (IgM, IgG, and IgA) against the Nucleoprotein (N). The test uses a recombinant protein representing the Nucleocapsid (N) antigen to aid in the detection of anti-SARS-CoV-2 antibodies with high affinity and it is based on a double antigen sandwich assay. The development of Elecsys® Anti-SARS-CoV-2 immunoassays is based on the use of a ruthenium and tripropylamine (TPA) complex and it is based on a one-step sandwich principle. Roche Anti-SARS-CoV-2 provides a qualitative result with a sensitivity of 100.0% (95% CI 88.10–100.0) and a specificity of 99.81% (95% CI 99.65–99.91). Result are expressed as cut-off index (COI; signal of sample/cut-off); values ≥1.00 COI are considered positive (Farina et al., 2021). Main characteristics of the three above described methods are summarized in Table 1.

2.3. Statistical analyses

Data, correlation analysis and linear regression for all figures were performed using GraphPad PRISM (version 7; GraphPad Software Inc., San Diego CA, USA). Data were arranged in tables and calculations were made with Microsoft Excel software. We determined with GraphPad PRISM: the 95% confidence intervals and Cohen's kappa coefficient (κ) (a measure that considers the possibility of the agreement occurring by chance). We subsequently carried out sensitivity and specificity analyzes

Table 1
Characteristics of the methods.

	Panbio™ COVID-19 IgG/IgM Rapid Test Device (Abbott)	NovaLisa® SARS-CoV-2 IgG and IgM (NovaTec)	Elecsys® anti-SARS-CoV-2 (Roche)
Method	Immunochromatography	ELISA	ECLIA
Class of antibodies	IgG and IgM	IgG and IgM	IgTotal
Antigen	Recombinant N protein	Recombinant N protein	Recombinant N protein
Time	10–20 min	± 2 h	18 min
Test sample	Capillary whole blood, venous, serum or plasma	Serum or plasma	Capillary whole blood, venous, serum or plasma
Volume sample	−20 μl for capillary or venous blood sampling −10 μl for serum or plasma	10 μl	20 μl
Interpretation	Direct reading	Determination of a score to cut-off ratio. Results are expressed in NTU (NovaTec Units)	Determination of a score to cut-off ratio. Results are expressed in COI (signal of sample/cut-off)

for true-negative and true-positive samples over all the tests performed. We report square R of coefficients of determination for association among continuous variables. Wilcoxon-sign-rank tests, followed by Spearman test were applied for comparison of paired samples and we considered Spearman r coefficient to evaluate the significatively of *p*-value. Spearman coefficient values are interpreted as follows: from 0.3 to 0.5 as low, from 0.5 to 0.75 as moderate/good and from 0.75 to 1.00 as excellent. A p-value <0.05 was considered as statistically significant.

3. Results

3.1. Breakdown analysis of tests

Results are summarized in Table 2 where the antibodies reactivity to N SARS-CoV-2 Ig (IgM, IgG and IgT) obtained with the three different methods are reported for each patient.

Regarding the results interpretation it should be highlighted that: Panbio™ COVID-19 IgG/IgM Rapid Test is a qualitative test, NovaLisa gives quantitative data in NTU (NovaTec Units) while Elecsys®Anti-SARS-CoV-2 results are expressed in COI (signal of sample/cut-off). In this contest, the only method in which it is possible to detect equivocal values is the ELISA one.

As reported in Table 2, the analysis of serological tests on sera from the 29 patients referring COVID-19 infection showed SARS-CoV-2 serology evidence in 28 cases; only one patient didn't show anti-N antibodies. In 19 cases concordant IgT positive results for all used tests were detected. Three sera showed positivity for both Panbio™ and Elecsys® IgT, one serum showed positive IgT results for Elecsys® and NovaLisa®, while in 4 sera only Elecsys® IgT were detected. Above sera concerning the 41 patients not showing COVID-19 symptoms in last year, only 12 (29%) resulted negative to all serological used tests while in 29 (71%) anti-N antibody was revealed. Among the positive results in 8 samples only anti-N IgM were detected.

3.2. Qualitative comparison

To qualitatively compare the analytical performances of the three methods, we carried out analyses in pairs by correlating the results obtained in the study (Table 3).

To compare data obtained by the two assays that detect separately IgG and IgM with those obtained by Elecsys®Anti-SARS-CoV-2 that detect total Ig we extrapolated total Ig for each test on the basis of the single Ig class result: positivity or doubtfulness have been respectively attributed to samples with at least IgM or/and IgG positive or equivocal.

The comparison between NovaLisa® - Panbio™ and Elecsys® - Panbio™ showed a moderate agreement with each other (respectively Cohen's kappa coefficient $\kappa = 0.47$ and $\kappa = 0.52$) Table 3A-C; whereas the comparison between Elecsys® - NovaLisa® revealed a lowly correlation (Cohen's kappa coefficient $\kappa = 0.297$; Table 3**B**).

3.3. Correlation and comparison of NovaTec and Roche

Elecsys® and NovaLisa® N-SARS-CoV-2 serology assays were compared by correlating the quantitative values observed with the two platforms. Wilcoxon-sign-rank tests, followed by Spearman test indicated a significant statistical correlation between IgT/ Elecsys® vs IgG/ NovaLisa® (Spearman r value:0.6678) (p value<0.0001) (Fig. 1A). Instead, the analysis of IgT/ Elecsys® vs IgM/ NovaLisa® showed no significant statistical correlation (Spearman r value: 0.2544) (p value 0.0585) (Fig. 1B). Both of Spearman tests are represented with linear regression.

4. Discussion

To monitor the spread of COVID-19 *pandemic*, several diagnostic tools have been developed. Currently, real-time PCR assay is considered the gold-standard to diagnose severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (C.S.G.O.T.I.C.O.T.O. Viruses, 2020).

In the context of a health emergency, SARS-CoV-2 screening and diagnosis can be improved by introducing lateral flow tests (Budd et al., 2023; Afzal, 2020).

Moreover, with the progress of the severe acute respiratory syndrome Coronavirus 2 (SARS-CoV-2) pandemic, the use of specific serological tests that would highlight the presence of specific antibodies has become increasingly important for the clinical management of patients with COVID, for epidemiological studies on the impact of the pandemic in various populations as well as for the evaluation of the response to vaccination.

Approved serological immunoassays are now widely available, but there is significant discussion regarding the clinical utility of these tests, as well as their clinical and analytical performance characteristics for routine applications (Sidiq et al., 2020).

The main goal of serologic tests is to measure antibody response to viral infection, as well as to detect patients with evident clinical signs of the diseases but with a negative SARS-CoV-2 molecular test, or to identify asymptomatic patients (Higgins et al., 2021).

In addition, these serological tests provide clear indications on the real spread of the virus allowing a real estimate of infected subjects. The timing of the persistence of antibodies to N allows to improve the clinical management of the disease and to better plan vaccination protocols (Harley and Gunsolus, 2020).

One of the most abundant and crucial structural components of SARS-CoV-2 is the nucleocapsid (N) protein, a multifunctional protein highly conserved among coronavirus family members involved in viral genome packaging. As it happens for several viruses (i.e EBV), nucleocapsid proteins like N-protein, are highly immunogenic, making them efficient diagnostic tools for detection of viral infection (Kang et al., 2021; Bai et al., 2021; Dreyfus et al., 2018).

Due to the increasingly high vaccination rates, the N-based SARS-CoV-2 serological assays provide a valuable tool to differentiate subjects with previous natural SARS-CoV-2 infection from those who received vaccine, since all available vaccine formulations are directed against viral Spike (S) protein (Di et al., 2021).

To date, several serological tests are available for the evaluation of anti-N SARS-CoV-2 antibodies. However, data are not always comparable and each laboratory looks for the most reliable, efficient and cheap test. Thus, with the present study, we aimed to compare analytical performance of three different serological tests for SARS-CoV-2,

Table 2
Comparison of the anti N antibodies reactivity obtained by the three methods for each serum. Each row represents a serum sample (Sample), each column a serological test divided in IgG, IgM and IgT. The NovaLisa® IgG and IgM results are expressed in NTU (NovaTec Units), an arbitrary interpretation for the total antibodies (IgT) is given for Panbio™ and NovaLisa®; the IgT detected by Elecsys® are reported as COI. Patients 47, 51 and 52 have additional withdrawals at different times (marked with lowercase letters a, b, c).

		Panbio™			NovaLisa® (NTU)					Elecsys® (COI)	
Sample	COVID-19 infection	IgG	IgM	IgT	IgG		IgM		IgT	IgT	
1	−	+	−	+	1.79	−	11.13	+	+	0.09	−
2	+	+	−	+	5.84	−	11.21	+	+	5.89	+
3	−	+	−	+	1.30	−	10.14	+/−	+/−	0.08	−
4	+	+	−	+	3.40	−	6.37	−	−	14.68	+
5	−	−	+	+	3.46	−	7.65	−	−	0.08	−
6	−	−	−	−	3.86	−	9.05	+/−	+/−	0.08	−
7	+	+	−	+	5.34	−	10.97	+/−	+/−	28.22	+
8	−	+	−	+	4.08	−	11.73	+	+	0.08	−
9	−	+	−	+	3.30	−	12.97	+	+	1.10	+
10	−	+	−	+	2.69	−	7.93	−	−	11.87	+
11	−	+	−	+	7.37	−	6.12	−	−	41.67	+
12	−	+	−	+	6.22	−	8.74	−	−	0.87	−
13	+	+	−	+	22.57	+	11.41	+	+	20.97	+
14	−	−	+	+	4.60	−	12.44	+	+	0.32	−
15	+	+	−	+	30.78	+	10.93	+/−	+	34.42	+
16	−	+	−	+	5.55	−	7.98	−	−	34.29	+
17	−	+	−	+	3.73	−	9.87	+/−	+/−	0.59	−
18	−	+	−	+	4.40	−	13.84	+	+	8.80	+
19	−	+	−	+	4.91	−	9.96	+/−	+/−	75.01	+
20	−	−	−	−	3.29	−	10.22	+/−	+/−	0.22	−
21	+	+	−	+	5.98	−	10.26	+/−	+/−	14.70	+
22	−	+	−	+	11.12	+	11.72	+	+	119.40	+
23	−	−	−	−	2.98	−	7.64	−	−	0.08	−
24	+	+	−	+	8.80	−	9.64	+/−	+/−	35.69	+
25	+	+	−	+	18.07	+	7.68	−	+	33.11	+
26	+	+	−	+	24.36	+	9.89	+/−	+	104.40	+
27	+	+	−	+	10.97	+/−	6.27	−	+/−	58.96	+
28	+	−	−	−	4.35	−	7.03	−	−	38.92	+
29	+	+	−	+	9.86	+/−	9.66	+/−	+/−	114.90	+
30	+	−	−	−	3.35	−	11.52	+	+	13.26	+
31	+	+	−	+	4.01	−	7.16	−	−	79.75	+
32	+	−	+	+	9.03	+/−	14.40	+	+	13.34	+
33	−	+	−	+	4.21	−	7.89	−	−	2.33	+
34	−	−	−	−	2.70	−	5.42	−	−	0.08	−
35	−	−	−	−	1.92	−	9.05	+/−	+/−	0.08	−
36	−	−	−	−	3.00	−	5.15	−	−	0.08	−
37	−	+	−	+	2.99	−	7.09	−	−	0.09	−
38	−	−	+	+	3.85	−	13.68	+	+	0.19	−
39	−	+	−	+	4.63	−	10.80	+/−	+/−	0.10	−
40	−	−	−	−	4.53	−	5.99	−	−	0.08	−
41	−	+	−	+	4.47	−	5.82	−	−	66.89	+
42	−	−	−	−	2.29	−	8.09	−	−	0.08	−
43	+	+	−	+	3.95	−	9.20	+/−	+/−	24.93	+
44	−	+	−	+	29.17	+	10.61	+/−	+	144.70	+
45	−	+	−	+	4.95	−	10.91	+/−	+/−	93.91	+
46	+	+	−	+	14.88	+	15.93	+	+	0.45	−
47a	+	+	−	+	9.76	+/−	6.23	−	+/−	7.74	+
47b		+	−	+	26.69	+	4.69	−	+	13.31	+
47c		+	−	+	4.45	−	7.21	−	−	11.49	+
48	+	+	−	+	31.71	+	3.26	−	+	29.08	+
49	−	−	−	−	45.61	+	3.82	−	+	0.17	−
50	+	+	−	+	20.19	+	12.80	+	+	47.10	+
51a	+	+	−	+	30.86	+	6.66	−	+	78.38	+
51b		+	−	+	17.68	+	11.20	+	+	38.06	+
52a	+	−	−	−	8.26	−	7.12	−	−	4.16	+
52b		+	−	+	6.93	−	5.43	−	−	2.85	+
53	−	+	−	+	22.13	+	4.84	−	+	79.29	+
54	+	+	−	+	22.17	+	8.66	−	+	9.65	+
55	+	+	−	+	2.91	−	3.00	−	−	1.35	+
56	−	−	−	−	3.08	−	2.60	−	−	0.67	−
57	−	+	−	+	4.68	−	3.47	−	−	72.41	+
58	+	−	−	−	2.59	−	2.56	−	−	5.80	+
59	+	+	−	+	14.16	+	10.52	+/−	+	10.66	+
60	−	+	−	+	25.24	+	13.61	+	+	55.62	+
61	−	−	−	−	2.86	−	2.30	−	−	4.21	+
62	−	+	−	+	19.33	+	7.55	−	+	123.10	+
63	−	−	−	−	2.59	−	2.36	−	−	0.08	−
64	−	−	−	−	3.25	−	3.52	−	−	0.07	−
65	−	−	−	−	2.18	−	0.83	−	−	0.15	−
66	−	−	−	−	3.06	−	1.41	−	−	0.06	−

(*continued on next page*)

Table 2 (*continued*)

		Panbio™			NovaLisa® (NTU)					Elecsys® (COI)	
Sample	COVID-19 infection	IgG	IgM	IgT	IgG		IgM		IgT	IgT	
67	–	–	–	–	5.43	–	2.76	–	–	0.09	–
68	–	–	–	–	2.15	–	0.68	–	–	0.06	–
69	+	+	–	+	11.23	+	10.23	+/–	+	19.04	+
70	+	–	–	–	2.33	–	1.47	–	–	0.08	–

Table 3
Qualitative comparison of results by different techniques. Cohen's kappa coefficient κ = A) 0.47; B) 0.297; C) 0.52. κ values were classified as almost perfect (0.8–1.0), substantial (0.6–0.8), moderate (0.4–0.6), fair (0.2–0.4), or poor correlations (<0.2).

A		Panbio™	
k = 0.47		**IgT (+)**	**IgT (−)**
NovaLisa®	**IgT (+)**	26	2
	IgT (−)	14	17

B		NovaLisa®	
k = 0.297		**IgT (+)**	**IgT (−)**
Elecsys®	**IgT (+)**	22	15
	IgT (−)	6	16

C		Panbio™	
k = 0.52		**IgT (+)**	**IgT (−)**
Elecsys®	**IgT (+)**	41	5
	IgT (−)	9	17

employing manual and automated procedures.

In this study, we analyzed anti-N antibodies measured by immunochromatographic test Panbio™ COVID-19 IgG / IgM Rapid Test Device by Abbott, Enzyme-Linked Immunosorbent Assays (ELISA) NovaLisa® SARS-CoV-2 (COVID-19) IgM and IgG by NovaTec and electrochemiluminescent immunoassay (ECLIA) Elecsys® anti-SARS-CoV-2 by Roche.

Firstly, we qualitatively compared the analytical methods, and we observed a moderate agreement between Elecsys® - Panbio™ and NovaLisa® - Panbio™ systems (respectively Cohen kappa coefficient and κ = 0.52 and κ = 0.47).

Thereafter, our results showed the validity of the rapid Panbio™ COVID-19 IgG / IgM Rapid Test Device by Abbott even if the information obtained through this test is merely qualitative, the system is reliable, as reported by previous studies (Li et al., 2020; Dell'Aversana Orabona et al., 2022). It has several advantages: low-cost testing, speed (the results are ready in 10–20 min) and the possibility of performing the assay in a non-laboratory environment. It's important to underlie that decentralized testing relieves hospitals from analysis overload. Given the speed of execution and the low cost of the antigen test, the usefulness of this test would be appropriate in all situations that require a quick response (such as airports, train or bus stations and hospitals) (Linares et al., 2020).

Immunoglobulin M (IgM) are the first to appear in response to exposure with the antigen thus indicating a recent infection; immunoglobulin G (IgG) are the most abundant in the antibody response and constitute about 70–75% of the total immunoglobulins present in serum. The presence of specific IgG against viral antigen indicates that a previous or asymptomatic infection occurred between host and pathogen. Therefore, the possibility of differentiating IgM and IgG antibodies could provide information on the timing of viral infection (Gaebler et al., 2021).

Moreover, diagnostic sensitivities between the IgG, IgM, and total antibody are indistinguishable in SARS-CoV assays thus representing a limitation for the study as previously reported in 2020 by Sidiq et al.

Correlation analysis of semi-quantitative data obtained in the present study shows indicated a positive correlation between IgT (anti-N) results by Elecsys® and IgG (anti-N) by NovaLisa® (Spearman r value: 0.667; p value<0.0001). Instead, the analysis of IgT (anti-N) Elecsys® and IgM (anti-N) NovaLisa® showed no statistical correlation. However, the comparison between Elecsys® and NovaLisa® showed good agreement thus confirming the reliability of both methods despite testing different antibody classes.

This result is in agreement with the short term persistence of circulating IgM during seroconversion (Racine and Winslow, 2009).

In this regard, Zhou et al. studying the dynamic changes that occur during seroconversion in COVID-19 patients, they showed that following SARS-CoV-2 infection, IgM titer increases rapidly in the first 2 weeks, then persists for the next 1–2 weeks and decreases after four weeks (Zhou et al., 2021).

In summary, our study demonstrates that all the three assays investigated offer the possibility to detect anti-N antibodies providing different possibilities to get results. Therefore, the choice of the test is related to the type of information that is needed to manage patients outcome and follow up.

5. Conclusion

Despite the reliability of the three methods discussed in this work, the monitoring of N antibodies, performed with different analytical systems, could give rise to results that are not always consistent with each other. Therefore, to avoid such problems, it is important for patients who have contracted natural infection to evaluate the immune

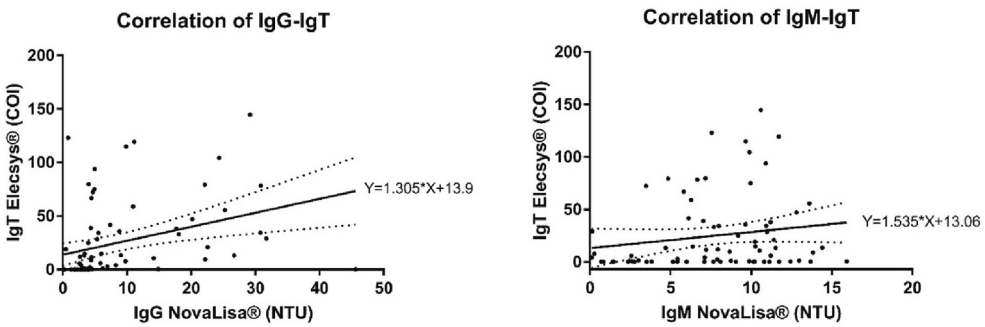

Fig. 1. Quantitative correlation analysis between Elecsys® and NovaLisa® tests. IgG NovaLisa® values (expressed in NTU) were compared to IgT Elecsys® values (expressed in COI) (panel a) (p value <0.0001). The dashed lines indicate the 95% confidence intervals (0,4451 to 0,7431). Correlation of the Elecsys® total Ig assay vs NovaLisa® IgM assay (panel b) was not statistically significant; the dashed lines indicate the 95% confidence intervals (−0,008067 to 0,4389). y = b + mx equations are indicated within each plot.

response through a single analytical platform.

CRediT authorship contribution statement

Aurelia Gaeta: Data curation, Investigation, Writing – original draft. **Antonio Angeloni:** Supervision, Writing – review & editing. **Anna Napoli:** Data curation, Resources. **Beatrice Pucci:** Formal analysis, Investigation. **Lilia Cinti:** Methodology. **Flavia Colaiacovo:** Data curation, Investigation, Writing – original draft. **Elena Berardelli:** Data curation, Validation. **Antonella Farina:** Project administration, Writing – review & editing. **Guido Antonelli:** Supervision. **Emanuela Anastasi:** Conceptualization, Funding acquisition, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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